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Characterizing a New Siphoviridae Bacteriophage CookieDog, and Phylogenetic Analysis of SSBP Families in Siphoviridae

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The Microbacterium phage CookieDog was discovered and isolated in late 2024 at Florida International University from samples collected at a marsh near Homestead, FL. The phage was isolated at a temperature of 30°C. Genome annotation was performed using Starterator, Glimmer, GeneMark, NCBI, PhagesDB, HHpred and Phamerator. DNA Master, Glimmer and GeneMark facilitated genome assembly and gene prediction; NCBI and HHpred provided detailed gene annotation, and Phamerator allowed for comparative genomic analysis with other bacteriophages to identify conserved genes and evolutionary relationships. CookieDog has a genome length of 38,761 bp, and it belongs to the GA cluster. Electron Microscopy shows a siphoviridae morphology. Bioinformatics analysis revealed a total of 65 genes, 33 of which have identified functions related to DNA replication, packaging, and structural proteins. The remaining 32 genes were annotated as hypothetical proteins, and their functions are unknown. A single 75 bp long tRNA was found from 30,428 to 30,502 bps. A translational frameshift was found within the tail assembly chaperone gene with a slippery sequence GGGAAAA at 11,135 bp. A unique feature about CookieDog and the cluster GA was the presence of a RecT-like DNA pairing protein that was only found in Cluster GA. An alignment was created for all Siphoviridae clusters containing known single-strand annealing proteins using Jalview and Clustal Omega. A phylogenetic tree was then constructed for the two families of single-strand annealing proteins, ERF and RecT using MEGA11. The tree reveals several distinct clusters containing both RecTs and ERFs. CookieDog contains a RecT that appears to be more closely related to ERF proteins than to other RecT proteins. Using Steve Notebooks we determined that 3 Phamilies were always shared in the genomes of phages containing ERF genes (clusters L and E) and the GA cluster (containing RecTs). The shared genes were Pham 180 (362) with unknown function, Pham 222741 (633) - HNH endonuclease and Pham 192284 (298) - DnaB-like Helicase. These genes were shared in phage clusters E and L (containing ERFs) and GA (containing RecTs), only varying in their downstream position in reference to the RecT/ERF gene. There are no Phams shared between members of the GA (containing RecTs) and other less related RecT containing pages (P, N, I1, I2, FJ, BV, AD). As more phage genomes are sequenced and annotated, they will contribute to a growing database that can assist future researchers in identifying gene functions and applications. Also, in the field of medicine, this increasing body of annotated phages offers greater potential for developing phage therapies to combat antibiotic-resistant infections, providing valuable alternatives for treating persistent bacterial diseases.